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X-ray Crystallography of the IP3 Receptor

I. Bosanac, J.-R. Alattia, F. Tong, and M. Ikura (U. of Toronto)

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Introduction: D-Myo-inositol 1,4,5-trisphosphate receptor (IP3R) is a pivotal molecule for cytosolic Ca^{2+} mobilization. The IP3 receptor (IP3R) behaves as a gated Ca2+ channel in response to IP3 production stimulated by extracellular signals such as hormones and neurotransmitters [1]. IP3 receptors are conserved in organisms spanning the evolutionary range from primitive slime mold to humans, and are widely expressed in almost all cell types. Different studies have shown that IP3R is involved in cell division, nerve growth, memory, learning and behavior [2-5]. Despite extensive studies on IP3R, the structural knowledge of this protein is very limited. A 3-D structure of the receptor is needed to understand the highly stereo specific binding of the IP3 ligand, to identify potential pharmacological targets and to help design biosensors to probe the IP3 signaling activity in the cell. Drug design, with the capability of promoting or inhibiting IP3R-induced Ca^{2+} release, clearly have considerable therapeutic potential.

Methods and Materials: The selenomethionyl derivative of the IP3 binding region was crystallized by the hanging drop method in space group $P2_1$ with unit cell dimensions a = 44.6 Å, b = 206.6 Å, c = 104.4 Å, and β = 100.8. The crystals were observed to be either thin plates or rods, with approximate dimensions 0.3 x 0.15 x 0.05 mm. Three sets of multiwavelength anomalous diffraction (MAD) experiments were performed using selenium as the anomalous scatterer. Each experiment was performed with a different crystal and recorded at three distinct wavelengths.

Results: A thorough search for the heavy atom positions using several software packages (SOLVE, CNS, SnB) was unsuccessful. The analysis of the diffraction data showed a number of complicating features, the most prominent being crystal decay due to x-ray damage. The prolonged exposure is dictated by the requirements of the space group and the high mosaicity observed for certain crystal orientations. In addition, there was evidence in some diffraction images for the presence of a minor lattice. Although the indexing software was able to identify the major lattice correctly, it is expected that the intensity of certain reflections would be erroneously estimated. Finally, there are serious indications that all crystals used in this study could be affected by pseudo-merohedral twinning.

Conclusions: The strategy for determining the structure of the IP3 receptor should be reviewed in light of the present results. Modifications to the approach will be sought at the data analysis level and eventually in the crystallization procedure.

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